IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2023 Vol.:27, Issue:1

Phytochemistry and Pharmacological Activity on the Fruits of *T. dioica* for Urolithiasis Action

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Anita Yadav*, Jitendra Kumar Malik

Institute of Pharmacy, P.K. University, Thanra, Shivpuri, M.P., India

Submitted: 25 March 2023
Accepted: 31 March 2023
Published: 30 April 2023

Keywords: *T. dioica*, TLC, Column Chromatography, Urolithiasis

ABSTRACT

Aim: This study aimed to investigate the effect of plant *T. dioica* used in traditional medicine on the dissolution of calcium oxalate stones. Then, the ability of effective plants to dissolve stones was investigated. **Methods:** The Fruit of the plant was extracted by the Soxhlet apparatus method. Preliminary phytochemical investigation confirmation for the secondary metabolites like alkaloids, flavonoids, and glycosides. Coloum chromatography was used for the isolation of the flavonoid compound. The isolated compound was interpreted by UV, FTIR, and NMR spectroscopy. The synthesized stones in the laboratory were incubated with different concentrations of the extract. Next, the concentrations of calcium oxalate were measured by a calcium kit. **Results:** The highest dissolution of calcium oxalate stones were observed by the *T. dioica* extract and the highest dissolution of calcium phosphate stones. The dissolution percentage of clinical stones by the *T. dioica* extract was significantly higher. The highest TD1 was observed in *T. dioica* extract. **Conclusion:** The *T. dioica* extract exhibited the greatest dissolution activity on laboratory calcium oxalate stones as well as clinical stones made of high amounts of calcium oxalate. Therefore, the extract can be effective in preventing and treating kidney stones.

INTRODUCTION:

Worldwide, the spread of kidney stone disease is rising with a few numbers of effective drugs. This urological disorder affects about 12% of the world's population [1]. About 80% of all kidney stones are composed of oxalate calcium and around 20% of kidney stones are made of calcium phosphate which, like oxalate calcium stones, is formed as a result of increased calcium [2, 3]. Due to the stupendous costs and side effects of instrument embedding and urinary tract surgery, special attention has now been drawn to the use of herbal products [4,5,6]. Medicinal plants have long been used as an important source by humans and even animals. [7, 8, 9]

Trichosanthes, a genus of the family Cucurbitaceae, is an annual or perennial herb distributed in tropical Asia, Polynesia, and Australia. [10] Over 20 species are recorded in India of which two, namely *T. anguina* and *T. dioica*, are cultivated as a vegetable. Other important species found throughout the world are *T. palmata, T. cordata, T. nervifolia, T. cucumerina, T. wallichiana, T. cuspida, T. incisa, T. laciniosa, T. kirilowii*, etc. *Trichosanthes dioica*, also referred to as "Sespadula" in English and "Parwal" in Hindi, is found abundantly throughout India. [11, 12] *T. dioica* leaf juice is used as a febrifuge, tonic in alopecia, and subacute liver enlargement instances. Leaf and fruit remedies for drunkenness and jaundice are mentioned in the Charaka Samhita. [13] The immature fruits are eaten fried and as dorma with roe stuffing, as well as used as a vegetable in soup, stew, curry, and sweet dishes. [14] Apart from the fruits, other parts of the plant, such as the leaves and tender shoots, have been used in traditional medicine since ancient times. When shade-dried fruits were mixed in the food of nondiabetic animals, specific medicinal properties such as hypocholesterolemic, and hypoglyceridimic were discovered. Its seeds and leaves have recently been discovered to be anti-diabetic agents. Numerous pharmaceutical studies have scientific research on several *T. dioica* components, but some other historically significant therapeutic uses are also now scientifically unproven. The plant can be used as an anti-inflammatory, anti-cancer, hypolipidemic, cardiotonic, diuretic, ulcer preventive, antidiabetic, etc. [15, 16, 17,18] The plant shows good antioxidant activity. The different chemical components that are present in the plant are vitamin A, vitamin C, tannins, saponins, alkaloids, peptides, tetra and pentacyclic triterpenes, etc. [19]

Given the richness of the traditional medicine and medicinal plants frequently cited in traditional medicine, this study was conducted to investigate *T. dioica* plants used in traditional medicine on the dissolution of calcium oxalate stones in vitro.

MATERIALS AND METHODS

Plant: First, the studied plant *T. dioica* was prepared from the local market, and after scientific and systematic identification by a botanist, a voucher herbarium specimen was deposited for each of them at the PK University, Dinara, MP.

Extraction: The powdered plant material (50g) was extracted by the Hot continuous Soxhlet extraction method. The plant material was extracted with Ethanol (99.9% v/v) (500ml) and Petroleum ether (500ml) for four days in a Soxhlet apparatus. The resulting solution was concentrated in a vacuum by a rotary evaporator, and the extracts were dried by freeze-drying. [20]

Preliminary Phytochemical Studies: Phytochemical tests were done in plant extracts for the detection of the presence of different chemical constituents such as; alkaloids, glycosides, flavonoids, essential oils, carbohydrates, proteins, tannins, and other substances which are responsible for biological activity. So the chemical tests are performed in the ethanolic and petroleum ether extract of *T. dioica*. For the detection of different chemical constituents was observed in Table 1. [21]

Column chromatography: Column chromatography is separated into two categories depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity or percolation, it is called gravity column chromatography. If the solvent is forced down the column by the air pressure, it is called flash chromatography. Data of column chromatography ethanolic extract of *T. dioica* Linn. Fruits were in Table 2. [22, 23]

IN VITRO CALCIUM OXALATE CRYSTALLIZATION INHIBITION:

Sample preparation: The various extraction of plant material was ready to use.

Experiment: The precipitation of calcium oxalate at 37 °C and pH 6.5 has been studied by the measurement of turbidity at 620nm. A spectrophotometer UV/Vis (SHIMADZU 1800) was employed to measure the developed turbidity due to the formation of calcium oxalate.

Procedure:

Study without inhibitor: The solutions of $CaCl_2.2H_2O$ (10mM) and $Na_2C_2O_4$ (4mM) were prepared using sodium chloride solution (0.15M). A volume of 1.5ml of calcium chloride dehydrate was transferred into the cell and blank reading was taken. 1.5ml of sodium oxalate solution was added to the previous volume, and the turbidity measurement was immediately started for a period of 10min. For each experiment, six replicates were taken.

Study with an inhibitor: The inhibitor (100%) was prepared by taking 0.5g of the extract with 60 ml of sodium chloride (0.15mM). From this inhibitor, prepare diluted inhibitory solutions (10%, 50%) using a solvent such as sodium chloride solution (0.15mM). A mixture of 1ml of calcium chloride dehydrate (10mm) and 1ml of the inhibiting solution was versed in the cell. Take a blank reading, and then a volume of 1ml of sodium oxalate (4mM) was added and the measurement was immediately started for 10 minutes.

The % of inhibition was calculated using the following formula:

$$I\ (\%) = [1\text{-}Ti/\ Tc] \times 100$$

Where Ti is a turbidimetric slope with an inhibitor, Tc is a turbidimetric slope without an inhibitor.

Microscopic study: The photographs were taken using a microscope optic equipped with a digital camera and connected to a microcomputer. At a time corresponding to the stage of growth and aggregation, a drop of the mixture of the crystallizable solution, or inhibitory solution was placed in the glass slide, which was immediately placed under the objective of the microscope. [24, 25]

RESULTS AND DISCUSSION

Extraction: The Percentage yield obtained was:

- Ethanol extract yield: 5.8 %w/w
- Petroleum ether extract yield: 3.2 %w/w

Preliminary Phytochemical Investigation: The chemical tests are performed in the ethanolic extract (EE) of *T. dioica* Linn. for the detection of different chemical constituents: The fruits of the plant were extracted with ethanol and then petroleum ether extract of fruits

extract of *T. dioica* Linn. was subjected for phytochemical screening for the detection of various plant constituents it is found that flavonoids compounds are present as a major active principle.

Table no 1: Data for the preliminary phytochemical screening of the whole plant of *T. dioica*

S. No.	Phytoconstituents	Ethanol Extract	Pet. ether Extract
1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Tannins And Phenolic	-	+
4.	Terpenoids	+	-
5.	Glycosides	+	-
6.	Saponins	-	+
7.	Protein	+	+
8.	Mucilage	-	-
9.	Carbohydrates	+	+
10.	Phytosterols	-	-

Column Chromatography: The Column Chromatography of isolated compounds from the ethanolic extract of *T. dioica* Linn. was carried out with hexane (100) to toluene: ethyl acetate and stationary phase silica gel. One fraction was collected from TD1 and TD2 on performing the TLC of fraction TD1 and TD2 were showing one spot respectively. Since fractions TD1 and TD2 showed only one spot so it was further investigated for 1H-NMR, FTIR, and UV Spectra& structure was proposed. The comparison of IR, NMR, and UV spectra of standard with isolated compound, indicated that this compound may be a flavonoid derivative having Quercetin structure as a proposed structure.

Figure No 1: TLC plate of compound TDE1

Figure no 2: TLC plate of compound TDE2

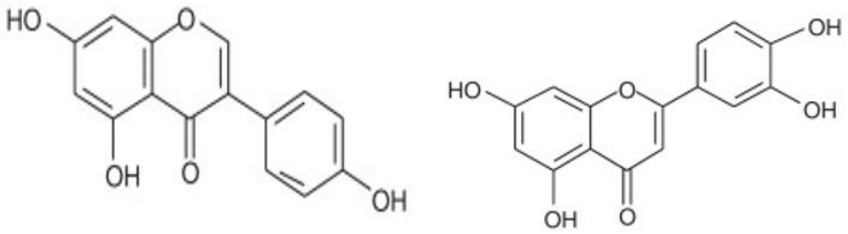
Table no 2: Data Showing the Column Chromatography Analysis

Fraction	Solvent system for TLC (ml)	Rf Value	The solvent used for crystallization	Color of the Comp.	Name of the Comp.
9, 10, 11	Hexane: ethyl acetate	0.68	Chloroform	Light Yellow	TDE1
21, 22, 23	Hexane: ethyl acetate	0.45	Chloroform	Brown	TDE2

Compound TDE1 showed greenish violet in appearance which is in the solid state. The melting point of this compound was 160-162˚C. It was soluble in chloroform and alcohol. On TLC, TDE1 showed a single spot having the solvent system of Hexane: Ethyl acetate (8:2). The Rf value of this compound was 0.68. Compound TDE2 showed dark green color in appearance which is a solid state. The melting point was 173-175˚C. Soluble in chloroform, a

single spot was obtained for this compound on TLC having the solvent system of Hexane: Ethyl acetate (8: 2) having the Rf value of 0.45.

The above data showed that this compound TDE2 may be a Flavonoid type of compound which was further confirmed by a chemical test [16].



Compound TD1 **Compound TD2**

Figure no 3: Flavonoid derivatives compound isolated from plant *T. dioica*

INVITRO ANTIUROLITHIATIC ACTIVITY:

Calcium Oxalate Crystallization Inhibition: The effect of ethanol extract of plant extract on various phases of calcium oxalate crystallization was determined by time course measurement of turbidity in the sodium chloride solution. The absorbance according to the time for tries without and with inhibitor was represented in the graph taking time vs. absorbance. The values of the change in absorbance for the extract were noted and a graph was plotted.

The graph shows an initial detectable increase in the turbidity after induction of the crystallization with sodium oxalate, which was observed. In the control experiment, there was an initial steep rise in turbidity (the nucleation phase), on attaining its maximum, it was followed by a decrease (the aggregation phase).

The ethanol and petroleum ether plant extract inhibited the slope of turbidity followed by a very slow decrease in the graph. Crystallization was inhibited significantly with ethanolic plant extract followed by petroleum ether plant extract. The linear proportion of the graph was taken up for detecting the slope.

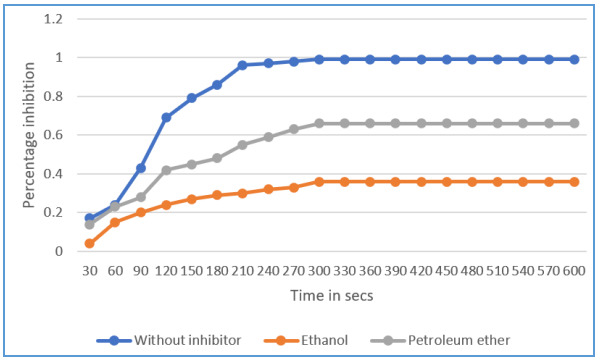


Figure no 4: Calcium oxalate crystallization inhibition with the formulation

Microscopic Study: The ethanolic plant extract being examined under the microscope show that the ethanolic plant extract had decreased the number of crystals. The first photograph corresponds to the stages of the growth and aggregation for the crystallization without the inhibitor of calcium oxalate. The comparison of photographs (II and III) of the tries with the inhibitor (ethanolic extract) enabled us to understand that crystals were greatly reduced in number, which explains that the ethanolic extract produces a significant quantity of inhibition on the growth of the crystal.

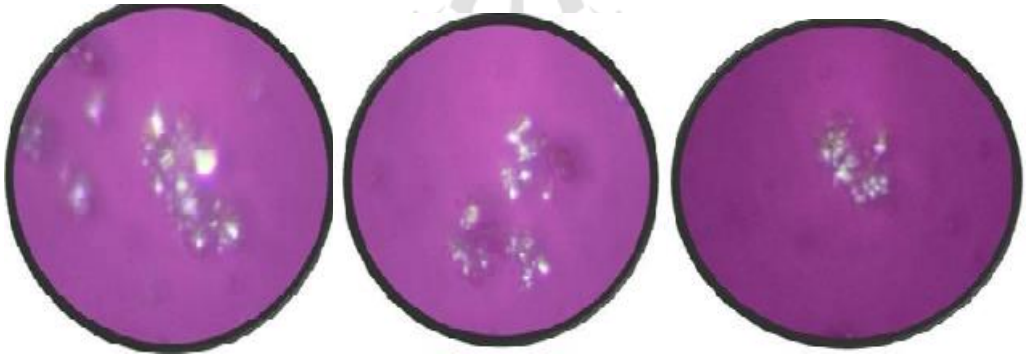


Figure no 5: Microscopic photographs of calcium oxalate crystallization inhibition

The photograph indicates that in cases where the ethanolic plant extract was not added, the number of crystals is maximum (photo I). In photographs II, and III which represent the crystal formation when the solution was mixed with plant extract, there was a clear indication that there is a dose-dependent manner of crystals formed with time.

Kidney oxalate stone is the result of the supersaturation of urine with certain urinary salts such as calcium oxalate. Since crystallizable oxalate species are pH dependent, the crystallization of oxalate in the absence of an inhibitor, led to the formation of calcium

oxalate monohydrate monitored by a light microscope, the process of calcium oxalate crystallization in control without the addition of inhibitors is shown in (Figure 5).

In the crystal growth experiments showing nucleation, growth, and aggregation, the rate of crystallization is usually controlled by the number of crystals of calcium oxalate as a function of time, following the introduction of seed crystals. Entitled constant volume against time in the composition calcium oxalate experiments determined that the rate of growth of crystals was made in the absence and presence of ethanolic plant extract.

To assess the inhibiting potential of substances for oxalate crystallization and understand the mechanisms of action of these inhibitors on oxalate crystallization steps viz. nucleation, growth, and aggregation, we tested the effectiveness of ethanolic plant extract.

Regarding the Antilithiatic activity, the ethanol extract of leaf powder of the Tricosanthes dioica has shown better activity than the Petroleum ether extract. It also has significant activity when compared to the standard drug Cystone. This significant antilithiatic activity was evidenced by increased excretion of sodium and potassium salts as well as the volume of urine output hence it was concluded that Ethanol extract of leaf powder of *T. dioica* possesses antilithiatic action.

CONCLUSION:

In the present study, dried powder of *T. dioica* was subjected to extraction using ethanol and petroleum ether for the extraction. Some extract was reserved for preliminary phytochemical investigation and the rest was utilized for pharmacological screening. From the present study, we conclude that the preliminary phytochemical analysis of *T. dioica* indicated the presence of Alkaloids, Flavonoids, Proteins, Saponins, Terpenoids, Phytosterols, Carbohydrates, and Fatty acids. In-vitro Calcium oxalate crystallization inhibition study was evaluated. From this study, we conclude that the ethanol extracts compound TD1 and TD2 of *T. dioica* inhibit the calcium oxalate crystallization in the order of 82%, and 55% respectively.

REFERENCES:

1. Align T, Petros B. Kidney stone disease: an update on current concepts. Adv Urol. 2018;2018:1–12.
2. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease. Philadelphia: Elsevier Saunders; 2005.
3. Moe OW. Kidney stones: pathophysiology and medical management. Lancet. 2006;367(9507):333–44.
4. Ronco C, Bellomo R, Kellum JA, Ricci Z. Critical Care Nephrology E-Book. Philadelphia: Elsevier Health Sciences; 2017.

5. Rosa M, Usai P, Miano R, Kim FJ, Agrò EF, Bove P, Micali S. Recent finding and new technologies in nephrolithiasis: a review of the recent literature. BMC Urol. 2013;13(1):1–11.
6. Touhami M, Laroubi A, Elhabazi K, Loubna F, Zrara I, Eljahiri Y, et al. Lemon juice has protective activity in a rat urolithiasis model. BMC Urol. 2007;7(1):1–10.
7. Ali A. Ibn Sina (Avicenna): the prince of physicians. Ann Saudi Med. 2007;27(2):134–5.
8. Faridi P, Roozbeh J, Mohagheghzadeh A. Ibn-Sina's life and contributions to medicinal therapies of kidney calculi. Iranian J Kidney Dis. 2012;6(5):339–45.
9. Tavakolifar B, Rezazadeh S, Badi H, Akhondzadeh S, Heidari M, Aghamohamadi E. The study of arnebia (Arnebiaeuchroma (Royle) IM Johnst) solution effectiveness on the treatment of Acne vulgaris. J Med Plants. 2010;9(34):150–5.
10. Rai PK, Gupta SK, Srivastava AK, Gupta RK, Watal G, 2013. A scientifc validation of
11. antihyperglycemic and antihyperlipidemic attributes of Trichosanthes dioica. ISRN Pharmacol2013:473059.
12. Sason R, Sharma A, Kushwaha RK, 2016. Pharmacological and phytochemical properties ofpatola - Trichosanthes dioica. World J Pharm Med Res 2:156–159.
13. Seshadri, V.S., and V.A. Parthasarathy, 2002. Cucurbits. p. 493–629 In: T.K. Bose, J. Kabir T.K. Maity, V.A. Parthasarathy, and M.G. Som (eds.), Vegetable crops. Naya Prokash, Kolkata,India.
14. Shah BN, Seth AK, 2010. Pharmacological potential of Trichosanthes dioica—an edible plant.Hygeia J Drugs Med 2:1–7.
15. Sharma G, Pandey DN, Pant MC, 1990. The biochemical valuation of feeding Trichosanthesdioica Roxb. seeds in normal & mild diabetic human subjects. In relation to lipid profile. Ind.J. Physiol. Pharmacol., 34(2):140-148.
16. Sharmila BG, Kumar G, Rajasekara PM, 2007. Cholesterol-lowering activity of the aqueousfruit extract of Trichosanthes dioica Roxb (L.) in normal and streptozotocin diabetic rats. ClinDiagn Res 1:561–569.
17. Shivhare Y, Singh P, Rajak H, Patil UK, Pawar RS, 2009. Antioxidant potential ofTrichosanthes dioica Roxb (fruits). Pharmacogn J 1:258–262.
18. Shivhare Y, Singh P, Patel KK, Prashant S, 2010a. Preliminary phytochemical screening andantioxidant activity of petroleum ether extract of Trichosanthes dioica Roxb (fruits). Res JPharmacognPhytochem 2:155–158.
19. Shivhare Y, Singh P, Patil UK, 2010b. Healing potential of Trichosanthes dioica Roxb on burnwounds. Res J PharmacolPharmacodyn 2:2168–2171.
20. Bijauliya RK, Jain SK, Alok S, Dixit VK, Singh VK and Singh M: Macroscopical, microscopical and physico-chemical studies on leaves of Dalbergia sissoo Linn. (Fabaceae). Int J Pharm Sci Res 2017; 8(4): 1865-73.doi: 10.13040/IJPSR.0975-8232.8(4).1865-73.
21. Bahmani M, Rafieian-Kopaei M, Parsaei P, Mohsenzadegan A. The anti-leech effect of Peganum harmala L. extract and some anti-parasite drugs on Limnatisnilotica. Afr J Microbiol Res. 2012;6(10):2586–90.
22. Joshi V, Parekh B, Joshi M, Vaidya A. Herbal extracts of Tribulus terrestris and Bergenia ligulata inhibit growth of calcium oxalate monohydrate crystals in vitro. J Cryst Growth. 2005;275(1–2):e1403–8.
23. Bijauliya RK, Jain SK, and Alok S: Antilithiatic activity of ethanolic leaves extract of Dalbergia sissoo Linn. ethylene glycol induced lithiasis in rats. Int J Pharm Sci & Res 2018; 9(11): 4923-29. doi: 10.13040/IJPSR.0975-8232.9(11).4923-29.
24. Bensatal A, Ouahrani MR, Inhibition of crystallization of calcium oxalate by theextraction of Tamarix gallica. Urol Res 2008; 36: p.283–287.
25. Sathya M, Kokilavani R. Antiurolithiatic activity of ethanolic root extract of Saccharum spontaneum on glycolic acid induced urolithiasis in rats. JDDAT; 2012, 2(5), 86-89.